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**W-6507 Ingelheim am Rhein(DE)**(54) **HIV protease inhibiting agents.**

(57) Disclosed herein are compounds of the general formula X-A-B-C'-D-Y wherein X is a terminal group, for example, alkanoyl or phenylalkanoyl radicals, A is an amino acid residue having a lower alkyl or lower cycloalkyl side chain, e.g. Ala or val, B is a non-peptide linking unit, e.g. statyl or Pheψ[CH<sub>2</sub>NH]Leu, C' is an amino acid residue of α-amino-β-cycloalkylpropionic acid, glutamic acid or α-aminoadipic acid, or a related derivative thereof, D is an amino-acid residue, for example, Phe or Val, and Y is hydroxy, alkoxy, amino, alkylamino or dialkylamino. The compounds inhibit the activity of human immunodeficiency virus (HIV) protease and interfere with HIV induced cytopathogenic effects in human cells. These properties render the compounds used for combating HIV infections.

**EP 0 443 559 A2**

Field of the Invention

This invention relates to compounds exhibiting activity against particular retroviruses, to processes for producing the compounds, to pharmaceutical preparations thereof, and to the use of the compounds for the manufacture of a medicament for combating infections caused by the retroviruses.

Background of the Invention

During the last ten years, increasing attention has been focused on retroviruses. These viruses cause a variety of diseases in vertebrates, the most insidious to humans being immunodeficiencies and cancers. In 1983, a retrovirus, known as human immunodeficiency virus type 1 (HIV-1), was established as a causative agent for acquired immune deficiency syndrome (AIDS). This virus has become a pestilence of alarming proportion. More recently, the closely related virus, human immunodeficiency virus type 2 (HIV-2) has been identified as a second causative agent of AIDS. (Hereinafter, the term "HIV" is meant to include both HIV-1 and HIV-2 and any mutants thereof.)

Presently, several compounds are being evaluated in the clinic as possible therapeutic agents for AIDS. Another compound, 3-deoxythymidine (known also as zidovudine or AZT), has been shown in the clinic to decrease mortality and the frequency of opportunistic infections in AIDS patients. This latter compound is being used to manage certain patients with symptomatic HIV infections. However, in spite of some recent progress, the need for an effective therapy for AIDS still exists. For recent reviews, see R.A. Weiss in "Molecular Basis of Virus Disease", Symposium of the Society for General Microbiology, Vol. 40, Eds. W.C. Russel and J.W. Almond, University Press, Cambridge, UK, 1987, pp 167-192, and R.C. Gallo and L. Montagnier, *Scientific American*, 259,(4), 40 (1988).

One approach to finding agents having anti-HIV activity is to inhibit the action of HIV-encoded enzymes. This manner of inhibition interferes with the replication and propagation of the virus. Such an approach has been applied successfully in a search for inhibitors of the viral encoded enzyme, reverse transcriptase (RT). More explicitly, the previously noted zidovudine was found to inhibit RT which is required for viral replication. Subsequently, zidovudine was developed as an anti-HIV agent. Still more recently, this approach has been investigated using another HIV-encoded enzyme known as HIV protease as the target enzyme. In one instance, pepstatin A was found to inhibit the intracellular processing of the HIV gag proteins. See, S. Seelmeier et al., *Proc. Natl. Acad. Sci. USA*, 85,6612 (1988). However, the development of pepstatin A as an anti-HIV agent seems improbable in view of its multiple activities. Subsequently, M.L. Moore et al., *Biochem. Biophys. Res. Comm.*, 159, 420 (1989) and G.B. Dreyer et al., *Proc. Natl. Acad. Sci. USA*, 86, 9752(1989) reported on investigations showing the inhibition of HIV protease by hexapeptide and heptapeptide analogs. A.D. Richards et al., *FEBS Letters*, 247,113 (1989), also have reported that acetyl-pepstatin and an octapeptide analog inhibit HIV protease *in vitro*. Similarly, *in vitro* inhibition of HIV protease activity by modified peptide substrates, based on amino acid sequences of six to ten amino acids of the HIV polypeptide, has been reported by S. Billich et al., *J. Biol. Chem.*, 263, 17905 (1988) and by M. Kotler et al., *Proc. Natl. Acad. Sci. USA*, 85, 4185 (1988). The recent publication of patent applications, typically B. Weidmann, UK patent application 2,203,740, published October 26, 1988, I.S. Sigal et al., European patent application 337,714, published October 18, 1989 and D.J. Kempf et al., PCT patent application WO 89/10752, published November 16, 1989, disclosing peptide-like compounds with retroviral protease inhibition, is indicative of the increasing interest in this area.

The present application discloses a group of compounds which are potent inhibitors of HIV protease. Moreover, a capacity to inhibit HIV induced cytopathogenic effects in human cells has been demonstrated for many of the compounds. Such properties, together with the attributes of a relatively selective action and an apparent lack of toxicity, renders the compounds useful as agents for combating HIV infections.

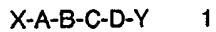
The compounds of this invention can be considered to have an incidental relationship to peptide derivatives since they have amino acid residues and/or derived amino residues incorporated into their structure and as such have a partial structural resemblance to pepstatin A and the previously noted peptide analogs. The present compounds also possess a partial structural resemblance to peptide derivatives reported to be renin inhibitors; for instance, see D.F. Veber et al., European patent application 77,028, published April 20, 1983, R.E. Ten Brink, PCT patent application WO87/02986, published May 21, 1987, P. Raddatz Australian patent application 73833/87, published December 17, 1987, J.P. Hudspeth et al., US patent 4,743,585, issued May 10, 1988, W.J. Greenlee et al., European patent application 273,696, published July 6, 1988, and A. Wagner et al., Australian patent application 76241/87, published February 4, 1988. The remaining structural features and differences in biological profiles distinguish the prior art compounds from the present peptide derivatives, notwithstanding the existence of broad generic disclo-

tures, such as found in the previously noted patent applications of Ten Brink and Sigal et al., encompassing an almost infinite number of compounds.

### Summary of the Invention

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The compounds of this invention are represented by formula 1



10 wherein X is

- (i) lower alkanoyl,
- (ii) lower alkanoyl monosubstituted with a hydroxy,
- (iii) alkanoyl, containing three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on different carbon atoms thereof,
- (iv) (lower alkoxy)carbonyl,
- (v) phenyl(lower alkanoyl),
- (vi) phenyl(lower alkanoyl) monosubstituted on the alkanoyl portion thereof with hydroxy, or
- (vii) phenyl(lower alkanoyl) monosubstituted at position 4 on the aromatic portion thereof with hydroxy, halo, lower alkyl, lower alkoxy, or  $\text{OCH}_2\text{C}(\text{O})\text{OL}$  wherein L is hydrogen, lower alkyl or benzyl;

20 A is  $\text{NHCH}(\text{R}^1)\text{CO}$  wherein  $\text{R}^1$  is lower alkyl or lower cycloalkyl;B is  $\text{NHCH}(\text{R}^2)\text{-Q}$  wherein  $\text{R}^2$  is

- (i) lower alkyl,
- (ii) lower cycloalkyl,
- (iii) (lower cycloalkyl)methyl,

25 (iv) phenyl or phenyl monosubstituted with hydroxy, lower alkyl or lower alkoxy, or

(v) benzyl or benzyl monosubstituted on the aromatic portion thereof with hydroxy, lower alkyl or lower alkoxy, and

Q is  $\text{CH}(\text{OH})\text{CH}_2\text{C}(\text{O})$  or  $\text{CH}_2\text{NHCH}(\text{R}^3)\text{C}(\text{O})$  wherein  $\text{R}^3$  has the same meaning as defined hereinabove for  $\text{R}^2$ ;30 C' is  $\text{NHCH}(\text{R}^4)\text{C}(\text{O})$  wherein  $\text{R}^4$  is lower alkyl, (lower cycloalkyl)methyl,  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{C}(\text{O})\text{-T}$  or  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{CR}^9\text{R}^{10}\text{C}(\text{O})\text{-T}$  wherein  $\text{R}^5$  to  $\text{R}^{10}$ , inclusive, are each independently a hydrogen or lower alkyl and T is hydroxy, lower alkoxy or amino;D is  $\text{NHCH}(\text{R}^{11})\text{C}(\text{O})$  wherein  $\text{R}^{11}$  is phenylmethyl, lower alkyl, or lower alkyl monosubstituted with hydroxy or benzyloxy;

35 and

Y is hydroxy, lower alkoxy, amino, lower alkylamino or di(lower alkyl)amino;

or a therapeutically acceptable salt thereof.

A preferred group of the compounds of this invention is represented by formula 1 wherein

X is as defined hereinabove,

40 A is Ala, Abu, Val, Nva, Leu, Ile, Nle, Tbg or Cpg;

B is  $\text{NHCH}(\text{R}^2)\text{-Q}$  wherein  $\text{R}^2$  is lower alkyl, (lower cycloalkyl)methyl, benzyl, (4-hydroxyphenyl)methyl or (4-methoxyphenyl)methyl and Q is  $\text{CH}(\text{OH})\text{CH}_2\text{C}(\text{O})$  or  $\text{CH}_2\text{NHCH}(\text{R}^3)\text{C}(\text{O})$  wherein  $\text{R}^3$  has the same meaning as defined for  $\text{R}^2$  in the last instance;45 C' is  $\text{NHCH}(\text{R}^4)\text{C}(\text{O})$  wherein  $\text{R}^4$  is lower alkyl, (lower cycloalkyl)methyl,  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{C}(\text{O})\text{-T}$  wherein  $\text{R}^5$  to  $\text{R}^8$ , inclusive, are each hydrogen and T is hydroxy, methoxy or amino;

D is Phe, Ile or Ser(OBzl); and

Y is lower alkoxy or amino;

or a therapeutically acceptable salt thereof.

50 A more preferred group of the compounds are represented by formula 1 wherein X is as defined hereinabove. A is Ala, Val, Leu, Ile or Tbg, B is  $\text{NHCH}(\text{R}^2)\text{CH}(\text{OH})\text{CH}_2\text{C}(\text{O})$  wherein  $\text{R}^2$  is 2-methylpropyl, cyclopropylmethyl, cyclohexylmethyl or benzyl, C' is Val or Leu, D is Phe and Y is amino; or a therapeutically acceptable salt thereof.55 Another more preferred group of the compounds are represented by formula 1 wherein X is lower alkanoyl; lower alkanoyl monosubstituted with hydroxy; alkanoyl, containing three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on a different carbon atom thereof; or (lower alkoxy)-carbonyl; A is Ala, Val, Leu, Ile, Tbg or Cpg; B is  $\text{NHCH}(\text{R}^2)\text{CH}_2\text{NHCH}(\text{R}^3)\text{C}(\text{O})$  wherein  $\text{R}^2$  is benzyl and  $\text{R}^3$  is lower alkyl; C' is Val, Glu or Gln; D is Ile or Ser(OBzl) and Y is amino; or a therapeutically acceptable salt thereof.

A most preferred group of the compounds are represented by formula 1 wherein X is acetyl, (S)-2-hydroxy-1-oxopropyl, (S)-2,3-dihydroxy-1-oxopropyl, (S)-2-hydroxy-1-oxo-3-phenylpropyl, 1-oxo-3-phenylpropyl, 1-oxo-4-phenylbutyl, 1-oxo-5-phenylpentyl, 3-(4-hydroxyphenyl)-1-oxopropyl, 3-(4-methoxyphenyl)-1-oxopropyl, 3-[4(carboxymethoxy)phenyl]-1-oxopropyl or 3-[4-(benzyloxycarbonylmethoxy)phenyl]-1-oxopropyl, A is Val, Leu, Ile or Tbg, B is NHCH(R<sup>2</sup>)CH(OH)CH<sub>2</sub>CO  
 5 wherein R<sup>2</sup> is cyclohexylmethyl, C' is Leu, D is Phe and Y is amino; or a therapeutically acceptable salt thereof.

Another most preferred group of the compounds are represented by formula 1 wherein X is acetyl, 2-methyl-1-oxopropyl, (S)-2-hydroxy-1-oxopropyl or methoxycarbonyl, A is Ala, Val, Ile, Tbg or Cpg, B is  
 10 NHCH(R<sup>2</sup>)CH<sub>2</sub>NHCH(R<sup>3</sup>)C(O) wherein R<sup>2</sup> is benzyl and R<sup>3</sup> is 2-methylpropyl or 2,2-dimethylpropyl, C' is Val, Glu or Gln, D is Ile or Ser(OBzl) and Y amino; or a therapeutically acceptable salt thereof.

Included within the scope of this invention is a pharmaceutical composition for treating HIV infections in a human comprising a compound of formula 1, or a therapeutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

15 The scope of the invention includes as well the use of said compounds for the manufacture of a medicament for treating HIV infections in a human, comprising an effective amount of the compound of formula 1, or a therapeutically acceptable salt thereof.

Also included within the scope is the use of said compounds for the manufacture of a medicament for protecting human cells against HIV pathogenesis, comprising an anti-HIV effective amount of a compound  
 20 of formula 1, or a therapeutically acceptable salt thereof.

Processes for preparing the compounds of formula 1 are described hereinafter.

#### Details of the Invention

#### 25 GENERAL

The term "residue" with reference to an amino acid means a radical derived from the corresponding  $\alpha$ -amino acid by eliminating the hydroxyl of the carboxy group and one hydrogen of the  $\alpha$ -amino group.

In general, the abbreviations used herein for designating the amino acids and the protective groups are  
 30 based on recommendations of the IUPAC-IUB Commission of Biochemical Nomenclature, see European Journal of Biochemistry, 138,9 (1984). For instance, Val, Glu, Gln, Tyr, Ala, Ile, Phe, Ser and Leu represent the residues of L-valine, L-glutamic acid, L-glutamine, L-tyrosine, L-alanine, L-isoleucine, L-phenylalanine, L-serine and L-leucine, respectively. The symbol "Abu" represents the residue of 2(S)-aminobutyric acid. The symbol "Adp" represents the residue of 2(S)-aminoadipic acid. The symbols "Cpa" and "Cha" represent  
 35 the residues of 2(S)-amino-3-cyclopropylpropionic acid (L-cyclopropylalanine) and 2(S)-amino-3-cyclohexylpropionic acid (L-cyclohexylalanine), respectively. The symbols "Nle", "Nva" and "Tbg" represent the residues of 2(S)-aminohexanoic acid (L-norleucine), 2(S)-aminopentanoic acid (L-norvaline) and 2(S)-amino-3,3-dimethylbutyric acid, respectively. The symbol "Cpg" represents the residue of 2(S)-amino-2-cyclopentylacetic acid. The symbol "Tba" represents the residue of 2(S)-amino-4,4-dimethylpentanoic acid. The  
 40 symbol "Glu(OMe)" represents the residue of O<sup>5</sup>-methyl hydrogen glutamate.

The term "lower alkyl" as used herein, either alone or in combination with a radical, means straight chain alkyl radicals containing one to six carbon atoms and branched chain alkyl radicals containing three to four carbon atoms and includes methyl, ethyl, propyl, butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl and 1,1-dimethylethyl.

45 The term "lower alkanoyl" as used herein means straight chain 1-oxoalkyl radicals containing from one to six carbon atoms and branched chain 1-oxoalkyl radicals containing three to six carbon atoms and includes acetyl, 1-oxopropyl, 3-methyl-1-oxobutyl, 3,3-dimethyl-1-oxobutyl and 2-ethyl-1-oxobutyl.

The term "lower cycloalkyl" as used herein, either alone or in combination with a radical, means saturated cyclic hydrocarbon radicals containing from three to six carbon atoms and includes cyclopropyl,  
 50 cyclobutyl, cyclopentyl and cyclohexyl.

The term "lower alkoxy" as used herein means straight chain alkoxy radicals containing one to six carbon atoms and branched chain alkoxy radicals containing three to four carbon atoms and includes methoxy, ethoxy, propoxy, hexoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter radical is known commonly as tertiary-butyloxy.

55 The term "halo" as used herein means a halo radical selected from bromo, chloro, fluoro or iodo.

The term "amino" as used herein means an amino radical of formula -NH<sub>2</sub>. The term "lower alkylamino" as used herein means alkylamino radicals containing one to six carbon atoms and includes methylamino, ethylamino, propylamino, 1-methyl-ethylamino and 2-methylbutylamino. The term "di(lower

alkyl)amino" means an amino radical having two lower alkyl substituents each of which contains one to six carbon atoms and includes dimethylamino, diethylamino, ethylmethylamino and the like.

The symbol "Ph" represents a phenyl radical. Other symbols include Ac for acetyl, "4-HOPh" for 4-hydroxyphenyl, "4-(BzIOC(O)CH<sub>2</sub>,O)Ph for 4-(benzyloxycarbonylmethoxy)phenyl and "4-(HOC(O)CH<sub>2</sub>,O)Ph" for 4-(carboxymethoxy)phenyl.

With reference to the symbol B of general formula 1, the radical "-NHCH(R<sup>2</sup>)-Q-" wherein R<sup>2</sup> is as defined hereinabove and Q is CH(OH)CH<sub>2</sub>C(O) represents the radical derived from the amino acid known as statine (i.e. 4(S)-amino-3(S)-hydroxy-6-methylheptanoic acid) and its close analogs. The radical is derived by eliminating the hydroxyl of the carboxy group and one hydrogen of the amino group of the corresponding  $\gamma$ -amino acid. Each such radical has two chiral centers and thus can exist in various optically active or optically inactive forms. All forms are included for the compounds of formula 1 and for the appropriate intermediates therefor, the 4(S)-amino-3(S)-hydroxy enantiomers being preferred. The requisite 4-amino-3-hydroxy pentanoic acids for preparing the synthon to incorporate the radical into the compound of formula 1 can be prepared by methods described by D.H. Rich and E.T.O. Sun, J. Med. Chem., 23, 27- (1980), and references therein.

The term "Sta" represents the radical -NHCH(2-methylpropyl)CH(OH)CH<sub>2</sub>C(O)-, derived from statine. The term "ACPPA" represents the radical NHCH(cyclopropylmethyl)CH(OH)CH<sub>2</sub>C(O)-, derived from 4-amino-5-cyclopropyl-3-hydroxypentanoic acid. The term "ACHPA" represents the radical -NHCH-(cyclohexylmethyl)CH(OH)CH<sub>2</sub>C(O)-, derived from 4-amino-5-cyclohexyl-3-hydroxypentanoic acid, and the term "AHPPA" represents the radical -NHCH(benzyl)-CH(OH)CH<sub>2</sub>C(O)-, derived from 4-amino-3-hydroxy-5-phenylpentanoic acid. The 4(S)-amino-3(S)-hydroxy enantiomers of these last four embodiments are preferred. Unless designated otherwise by an antecedent such as (3R, 4R), the terms Sta, ACPPA, ACHPA and AHPPA represent their respective 4(S)-amino-3(S)-hydroxy enantiomers.

Also note that when B is the radical "NHCH(R<sup>2</sup>)-Q" wherein R<sup>2</sup> is as defined hereinabove and Q is CH<sub>2</sub>NHCH(R<sup>3</sup>)C(O) wherein R<sup>3</sup> is as defined hereinabove, the radical is equivalent to two adjoining, corresponding amino acid residues (or derived amino acids residues) wherein the amide bond joining the two residues is reduced. According to convention, the latter radical can be expressed symbolically as two amino acid residues (in the three letter system) with the symbol " $\psi$ [CH<sub>2</sub>,NH]" inserted between the designation of the two adjoining amino acid residues. In this instance, the radical can be derived from  $\alpha$ -amino acids having the (R)- or preferably the (S)-chiral configuration for the  $\alpha$ -carbon atom. Accordingly, for example, the peptide of formula 1 wherein X is acetyl, A is Val, B is NHCH(benzyl)CH<sub>2</sub>NHCH(2-methylpropyl)C(O) with two (S)-asymmetric centers, C is Glu, D is Ile and Y is amino can be designated as Ac-Val-Phe $\psi$ [CH<sub>2</sub>NH]-Leu-Glu-Ile-NH<sub>2</sub>.

With reference to the compounds of formula 1, note that the asymmetric  $\alpha$ -carbon atoms of the amino acid residues or derived amino acid residues represented by the symbols A, C' and D have the S configuration, whereas asymmetric carbon atoms, residing in the side chain of these amino acid or derived amino acid residues also may have the R configuration.

The term "coupling agent" as used herein means an agent capable of effecting the dehydrative coupling of a free carboxy group of one reactant with a free amino group of another reactant to form an amide bond between the reactants. The agents promote or facilitate the dehydrative coupling by activating the carboxy group. Descriptions of such coupling agents and activating groups are included in general textbooks of peptide chemistry; for instance, E. Schroder and K.L. Lubke, "The Peptides", Vol. 1, Academic Press, New York, N.Y., 1965, pp 2-128, and K.D. Kopple, "Peptides and Amino acids", W.A. Benjamin, Inc., New York, N.Y., 1966, pp 33-51. Examples of coupling agents are thionyl chloride, diphenylphosphoryl azide, dicyclohexylcarbodiimide, N-hydroxysuccinimide, or 1-hydroxybenzotriazole in the presence of dicyclohexylcarbodiimide. A very practical and useful coupling agent is (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate, described by B. Castro et al., Tetrahedron Letters, 1219 (1975), see also D. Hudson, J. Org. Chem., 53, 617(1988), either by itself or in the presence of 1-hydroxybenzotriazole.

The term "pharmaceutically acceptable carrier" as used herein means a non-toxic, generally inert vehicle for the active ingredient, which does not adversely affect the ingredient.

The term "effective amount" as used herein means a predetermined amount of the compound of this invention sufficient to be effective against HIV in vivo.

## PROCESS

The compounds of formula 1 can be prepared by processes which incorporate therein methods commonly used in peptide synthesis such as the classical solution coupling of amino acid residues and/or

peptide fragments. Such methods are described, for example, by E. Schroder and K. Lubke, cited above, in the textbook series, "The Peptides: Analysis, Synthesis, Biology", E. Gross et al., Eds., Academic Press, New York, N.Y., 1979-1987, Volumes 1 to 8, and by J.M. Stewart and J.D. Young in "Solid Phase Peptide Synthesis", 2nd ed., Pierce Chem. Co., Rockford, IL, USA, 1984.

5 A common feature of the aforementioned processes for preparing the present compounds is the protection of any labile side chain groups of the reactants with suitable protective groups which will prevent a chemical reaction from occurring at that site until the protective group is ultimately removed. Usually also common is the protection of an  $\alpha$ -amino group on an amino acid or a fragment while that entity reacts at the carboxy group, followed by the selective removal of the  $\alpha$ -amino protective group to allow subsequent  
10 reaction to take place at that location.

Another feature of the preparation of the peptides of formula 1 is the incorporation into the peptide of the unit B. As noted previously the unit B can be either of two subunits, i.e.  $\text{NHCH}(\text{R}^2)\text{CH}(\text{OH})\text{CH}_2\text{C}(\text{O})$  wherein  $\text{R}^2$  is as defined herein or  $\text{NHCH}(\text{R}^2)\text{CH}_2\text{NHCH}(\text{R}^3)\text{C}(\text{O})$  wherein  $\text{R}^2$  and  $\text{R}^3$  are as defined herein. The first-mentioned subunit can readily be incorporated into the peptide by coupling the N-protected  
15 derivative of the corresponding 4-amino-3-hydroxypentanoic acid (of formula  $\text{NH}_2\text{CH}(\text{R}^2)\text{CH}(\text{OH})\text{CH}_2\text{C}(\text{O})\text{OH}$ ), noted above, at the appropriate point during the preparation of the peptide by the classical methods of coupling of amino acid residues or fragments. The second-mentioned subunit,  $\text{NHCH}(\text{R}^2)\text{CH}_2\text{NHCH}(\text{R}^3)\text{C}(\text{O})$ , can be formed initially by a reductive alkylation between two sub-fragments, each subfragment containing a precursor portion of the B unit. For example, the reductive alkylation of H-Leu-OH p-nitrobenzyl  
20 ester with Boc-phenylalaninal in the presence of sodium cyanoborohydride gives Boc-Phe $\psi$ [ $\text{CH}_2\text{NH}$ ]Leu-OH nitrobenzyl ester having the subunit  $\text{NHCH}(\text{R}^2)\text{CH}_2\text{NHCH}(\text{R}^3)\text{CO}$  in which  $\text{R}^2$  is benzyl and  $\text{R}^3$  is 2-methylpropyl. Alternatively, a larger fragment of the compound of formula 1 can be obtained by an analogous reductive alkylation wherein at least one of the subfragments contains one or more of the other units to be incorporated.

25 More specifically, the compounds of formula 1 can be prepared by: coupling an acid of the formula  $\text{X}^1\text{-OH}$  wherein  $\text{X}^1$  is lower alkanoyl; a lower alkanoyl monosubstituted with a hydroxy; an alkanoyl of three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on different carbon atoms thereof; a phenyl(lower alkanoyl); a phenyl(lower alkanoyl) monosubstituted on the alkanoyl portion thereof with a hydroxy; or a phenyl(lower alkanoyl) monosubstituted at position 4 on the  
30 aromatic portion thereof with hydroxy, lower alkyl, lower alkoxy or  $\text{OCH}_2\text{C}(\text{O})\text{-OL}^1$  wherein  $\text{L}^1$  is lower alkyl or benzyl; with a key intermediate of formula  $\text{H-A-B-C}''\text{-D-Y}$  wherein A, B, D and Y are as defined herein and  $\text{C}''$  is the radical  $\text{NHCH}(\text{R}^{4A})\text{CO}$  wherein  $\text{R}^{4A}$  is lower alkyl, (lower cycloalkyl) methyl,  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{C}(\text{O})\text{-T}^1$  or  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{CR}^9\text{R}^{10}\text{C}(\text{O})\text{-T}^1$  wherein  $\text{R}^5$  to  $\text{R}^{10}$ , inclusive, are as defined herein and  $\text{T}^1$  is an O-(carboxy protective group), for example, benzyloxy, cyclohexyloxy or 2,6-dichlorobenzyloxy, or  $\text{T}^1$  is amino; followed, if required, by carboxy deprotection or by carboxy deprotection and esterification, to give the  
35 corresponding compound of formula 1. Note that the benzyl of  $\text{L}^1$  can serve a dual role, i.e. serve as a progenitor for the corresponding radical of the ultimate product of the process or serve as a carboxy protective group. Also, note that selective removal of a carboxy protective group, if required, can be effected by the choice of appropriate carboxy protective groups according to known methods.

40 Alternatively, the compounds of formula 1 wherein X is a phenyl(lower alkanoyl) monosubstituted on the aromatic portion thereof with  $\text{OCH}_2\text{C}(\text{O})\text{-OL}$  wherein L is as defined herein and A, B,  $\text{C}''$ , D and Y are as defined herein can be prepared by subjecting the intermediate of formula  $\text{X}^1\text{-A-B-C}''\text{-D-Y}$ , wherein  $\text{x}^1$  is a phenyl(lower alkanoyl) monosubstituted on the aromatic portion thereof with hydroxy and A, B,  $\text{C}''$ , and D are as defined herein, to O-alkylation with an alkylating agent of formula  $\text{K-CH}_2\text{C}(\text{O})\text{-OL}^1$  wherein K is  
45 bromo, chloro or iodo and  $\text{L}^1$  is as defined herein above, in the presence of a suitable strong base; followed, if required, by deprotection to obtain the desired corresponding compound of formula 1. Suitable strong bases for the above-noted alkylation include alkali metal carbonates, preferably potassium carbonate; alkali metal hydroxides, preferably sodium hydroxide or potassium hydroxide; or alkali metal hydrides, preferably sodium hydride.

50 The requisite intermediates for the preceding processes are prepared by stepwise coupling the appropriate units, e.g. A, B,  $\text{C}''$ , D and E according to known methods of peptide synthesis.

The compound of formula 1 of this invention can be obtained in the form of a therapeutically acceptable salt. In the instance where a particular peptide has a residue which functions as a base, examples of such salts are those with organic acids, e.g. acetic, lactic, succinic, benzoic, salicylic, methanesulfonic or p-toluenesulfonic acid, as well as polymeric acids such as tannic acid or carboxymethyl cellulose, and also  
55 salts with inorganic acids such as hydrohalic acids, e.g. hydrochloric acid, or sulfuric acid, or phosphoric acid. If desired, a particular acid addition salt is converted into another acid addition salt, such as a non-toxic, pharmaceutically acceptable salt, by treatment with the appropriate ion exchange resin in the manner

described by R.A. Boissonnas et al., *Helv. Chim. Acta*, 43, 1849 (1960). In the instance where a particular peptide has one or more free carboxy groups, examples of such salts are those with the sodium, potassium or calcium cations, or with organic bases, for example, triethylamine or N-methylmorpholine.

In general, the therapeutically acceptable salts of the peptides of formula 1 are biologically fully equivalent to the peptides themselves.

## BIOLOGICAL ASPECTS

The HIV protease inhibiting properties and the cell protective effect against HIV pathogenesis of the compounds of formula 1, or a therapeutically acceptable salt thereof, can be demonstrated by biochemical, microbiological and biological procedures.

A particular useful procedure for demonstrating the HIV protease inhibiting properties of the compounds of formula 1 or their therapeutically acceptable salts is the "Recombinant HIV Protease HLPC Assay". The procedure is based on the capacity of the test compound to inhibit enzymatic cleavage by HIV protease of a decapeptide (the substrate) having an amino acid sequence which includes a known HIV protease cleavage site of a HIV polyprotein; see H.G. Krausslich et al., *Proc. Natl. Acad. Sci. USA*, 86,807(1989). Details of this assay together with the results obtained for exemplified compounds of formula 1 are described in the examples hereinafter.

The capacity of the compounds of formula 1 or their therapeutically acceptable salts to protect cells against HIV infection can be demonstrated by microbiological procedures for evaluating the inhibitory effect of a test compound on the cytopathogenicity of HIV in human T4 cell lines. Typical of such procedures are those described by S. Harada and N. Yamamoto, *Jpn. J. Cancer Res. (Gann)*, 76,432(1985), and S. Harada et al., *Science*, 229,563 (1985). An assay based on the latter procedures is described in the examples hereinafter.

When a compound of this invention, or a therapeutically acceptable salt thereof, is used to combat HIV infections in a human, the peptide can be administered orally, topically or parenterally, in a vehicle comprising one or more pharmaceutically acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, the chosen route of administration and standard biological practice. For oral administration, the compound or a therapeutically acceptable salt thereof can be formulated in unit dosage forms such as capsules or tablets each containing a predetermined amount of the active ingredient, ranging from about 25 to 500 mg, in a pharmaceutically acceptable carrier. For topical administration, the compound can be formulated in a pharmaceutically acceptable vehicle containing 0.1 to 10 percent, preferably 0.5 to 5 percent, of the active agent. Such formulations can be in the form of a cream, lotion, sublingual tablet, or preferably a transdermal patch or buccal patch. For parenteral administration, the compound of formula 1 is administered by either intravenous, subcutaneous or intramuscular injection, in compositions with pharmaceutically acceptable vehicles or carriers. For administration by injection, it is preferred to use the compound in solution in a sterile aqueous vehicle which may also contain other solutes such as buffers or preservatives as well as sufficient quantities of pharmaceutically acceptable salts or of glucose to make the solution isotonic.

Suitable vehicles or carriers for the above noted formulations can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", 16th ed., Mack Publishing Company, Easton, Penn., 1980.

The dosage of the compound will vary with the form of administration and the particular active agent chosen. Furthermore, it will vary with the particular host under treatment. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

For oral administration, the compound or a therapeutically acceptable salt is administered in the range of 1.0 to 75 mg per kilogram of body weight per day, with a preferred range of 2.5 to 20 mg per kilogram. With reference to systemic administration, the compound of formula 1 is administered at a dosage of 10 mcg to 1000 mcg per kilogram of body weight per day, although the aforementioned variations will occur. However, a dosage level that is in the range of from about 50 mcg to 500 mcg per kilogram of body weight per day is most desirably employed in order to achieve effective results.

Although the formulations disclosed hereinabove are effective and relatively safe medications for treating HIV infections, the possible concurrent administration of these formulations with other antiviral medications or agents to obtain beneficial results is not excluded. Such other antiviral medications or agents include soluble CD4, zidovudine, dideoxycytidine, phosphonoformate, ribavarin or antiviral interferons (e.g.

$\alpha$ -interferon or interleukin-2).

The following examples illustrate further this invention. Solution percentages or ratios express volume to volume relationship, unless stated otherwise. Temperatures are given in degrees Celsius. Proton nuclear magnetic resonance (NMR) spectra were obtained at 200 MHz. Abbreviations used in the examples include

- 5 Boc: t-butyloxycarbonyl; BOP: (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; Bzl: benzyl; DMF: dimethyl formamide; Et<sub>2</sub>O: diethyl ether; EtOAc: ethyl acetate; EtOH: ethanol; HPLC: high performance liquid chromatography; MeOH: methanol; TFA: trifluoroacetic acid; THF: tetrahydrofuran; TLC: thin layer chromatography.

# 10 Example 1

N-[N-[N-[N-[3-(4-Hydroxyphenyl)propionyl]-L-valyl-4(S)-amino-3(S)-hydroxy-5-cyclohexylpentanoyl]-L-leucyl]-phenylalaninamide, (4-HOPh)CH<sub>2</sub>CH<sub>2</sub>CO-Val-ACHPA-Leu-Phe-NH<sub>2</sub>

- 15 a) Boc-Phe-NH<sub>2</sub>: Isobutyl chloroformate (2.86 mL, 22 mmol) was added dropwise at 0° to a stirred solution of N-methylmorpholine (2.42 mL, 22 mmol) and Boc-Phe-OH (5.31 g, 20 mmol) in dry THF. The resulting solution was stirred for an additional 30 min at 0°. Thereafter, a 28% aqueous solution of ammonia (5 mL) was added dropwise over 5 min. The solvent was evaporated and the residue was dissolved in EtOAc. The solution was washed successively with a 5% aqueous solution of citric acid (three times), saturated aqueous NaHCO<sub>3</sub> (three times) and saturated aqueous NaCl. The organic

20 solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to afford Boc-Phe-NH<sub>2</sub> (5.0 g, 94%) as a white solid.

- b) Boc-Leu-Phe-NH<sub>2</sub>: A solution of Boc-Phe-NH<sub>2</sub> (6.46 g, 24.4 mmol) in 6N HCl/dioxane (84 mL) was stirred at room temperature (20-22°) under a nitrogen atmosphere for 30 min. The solvent was evaporated and the residue was dried under high vacuum. The solid residue was suspended in dry acetonitrile (300 mL) and the resulting mixture was cooled to 0 - 5° and stirred under a nitrogen
- 25 atmosphere. Dry Et<sub>3</sub>N (3.7 mL, 26 mmol) was added, followed by Boc-Leu-OH(monohydrate) (5.4 g, 22 mmol), BOP (10.69 g, 24.2 mmol) and more Et<sub>3</sub>N (7.4 mL, 53 mmol). After 1.5 h, more BOP (4.28 g, 9.7 mmol) and Et<sub>3</sub>N (1.48 mL, 10.6 mmol) were added. The reaction was stirred for an additional 15 min, then the acetonitrile was evaporated under reduced pressure. The residue was partitioned between a saturated aqueous solution of NaCl (200 mL) and EtOAc (three times 200 mL). The combined organic
- 30 solutions were washed successively with 100 mL each of a 10% aqueous solution of citric acid, water, a 5% aqueous solution of NaHCO<sub>3</sub> (three times) and water. The organic solution was dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. Chromatography of the residue over silica gel (eluent = EtOAc) gave a white gum which was triturated with Et<sub>2</sub>O-hexane. The resulting solid was collected to afford Boc-Leu-Phe-NH<sub>2</sub> (7.7 g, 93%) as a white solid. Mass spectrum: 378 (M + H)<sup>+</sup>. Amino acid analysis: Leu, 1.00; Phe, 1.00.

- c) Boc-ACHPA-Leu-Phe-NH<sub>2</sub>: A solution of Boc-Leu-Phe-NH<sub>2</sub> (100 mg, 0.26 mmol) in 6N HCl/dioxane (1 mL) was stirred at room temperature under a nitrogen atmosphere for 15 min. The solvent was evaporated and the residue was dried under high vacuum for 1 h. The solid was suspended in dry acetonitrile (2 mL) and the suspension was stirred under a nitrogen atmosphere. The solution was
- 40 adjusted to pH 8 (wet pH paper) by the addition of N-methylmorpholine, then Boc-ACHPA-OH (83 mg, 0.26 mmol) was added. The solution was stirred at room temperature for 1 h (during which time pH 8 was maintained by the occasional addition of N-methylmorpholine). The mixture was poured into a saturated aqueous solution of NaCl. The aqueous solution was extracted twice with EtOAc. The combined organic extracts were washed successively with ice-cold 0.5 N aqueous HCl, 10% aqueous
- 45 Na<sub>2</sub>CO<sub>3</sub> (twice) and saturated aqueous NaCl (three times). The organic solution was dried (Na<sub>2</sub>SO<sub>4</sub>) and the solvent was evaporated. Chromatography of the residue over silica gel, eluting with 5% MeOH in chloroform, afforded Boc-ACHPA-Leu-Phe-NH<sub>2</sub> (138 mg, 92%) as a white solid.

- d) Boc-Val-ACHPA-Leu-Phe-NH<sub>2</sub>: By following the procedure of section c) of this example, Boc-ACHPA-Leu-Phe-NH<sub>2</sub> (72 mg, 0.13 mmol) was deprotected in 6N HCl/dioxane to give H-ACHPA-Leu-Phe-NH<sub>2</sub>
- 50 hydrochloride which in turn was coupled to Boc-Val-OH (27 mg, 0.13 mmol) with BOP to give a crude product (80 mg). Chromatography of the crude product on silica gel, using 5% MeOH in chloroform as the eluent, afforded Boc-Val-ACHPA-Leu-Phe-NH<sub>2</sub> (71 mg, 84%) as a white solid. Mass spectrum: 674.6 (M + H)<sup>+</sup> and 574.6 (M + H - Boc)<sup>+</sup>.

- e) (4-BzlOPh)CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-PheNH<sub>2</sub>: A solution of the latter product (101 mg, 0.15 mmol) in 6N HCl/dioxane (3 mL) was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the residue was dried under high vacuum for 15 min. The resulting solid was dissolved in acetonitrile (5 mL). N-methylmorpholine (99  $\mu$ L, 0.90 mmol), 3-[4-(benzyloxy)phenyl]-
- 55 propanoic acid [(4-BzlOPh)CH<sub>2</sub>CH<sub>2</sub>C(O)OH, 46.1 mg, 0.18 mmol] and BOP (79.6 mg, 0.18 mmol) were



added to the solution. After 2 h of stirring at room temperature, the solid material in the reaction mixture was collected by filtration and washed successively with several portions of water and Et<sub>2</sub>O. The resulting white solid was dried over P<sub>2</sub>O<sub>5</sub> under vacuum for 24 h to give (4-BzlOPh)CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub> (93.4 mg, 77%). Mass spectrum: 812 (M+H)<sup>+</sup>. Amino acid analysis: Leu, 0.97; Phe, 1.05; Val, 0.98.

f) The title compound of this example: The latter product (56 mg, 0.07 mmol) in a solution of MeOH (5 mL) was subjected to hydrogenolysis for 18 h at atmospheric pressure using 20% by weight of Pd(OH)<sub>2</sub> on carbon as the catalyst. The reaction mixture was filtered through a 45 μm membrane and the filtrate was concentrated under reduced pressure. The residue was purified by chromatography on silica gel (20 mL), using a gradient of 1 to 5% MeOH in chloroform as the eluent to give a white powder. Trituration of the powder with Et<sub>2</sub>O gave the title compound of this example (30.5 mg, 61%). Mass spectrum: 722 (M+H)<sup>+</sup>. Amino acid analysis: Leu, 0.94; Phe, 1.06; Val, 0.90.

#### Example 2

##### [4-(BzlOC(O)CH<sub>2</sub>O)Ph]CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>

The title compound of example 1 (89.2 mg, 0.12 mmol) was mixed with K<sub>2</sub>CO<sub>3</sub> (85 mg, 0.62 mmol) in dry dimethylformamide (5 mL). Benzyl bromoacetate (39 μL, 0.25 mmol) was added to the mixture. The mixture (a slurry) was stirred for 48 h at room temperature. The solvent was removed under reduced pressure. The residue was suspended in water (10 mL) and the suspension was subjected to sonication for 1 h. The solid was collected and dissolved in MeOH (30 mL). The solution was filtered and the filtrate evaporated under reduced pressure. The residue was triturated with Et<sub>2</sub>O and water, and then dissolved in dimethylsulfoxide (1 mL). Addition of water to the latter solution precipitated a white solid which after trituration with EtOAc afforded the title compound of this example (32 mg, 30%). Mass spectrum: 871 (M+H)<sup>+</sup>. Amino acid analysis: Leu, 1.00; Phe, 1.00; Val, 1.00.

#### Example 3

##### [4-(HOC(O)CH<sub>2</sub>O)Ph]CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>

The title compound of example 2 was subjected to hydrogenolysis according to the procedure of section f) of example 1. Filtration of the reaction mixture and concentration of the filtrate afforded a solid residue. The residue was triturated with Et<sub>2</sub>O to give the title compound of this example (14.3 mg, 81%). Mass spectrum: 780 (M+H)<sup>+</sup>. Amino acid analysis: Leu, 1.00; Phe, 1.01; Val, 0.99.

#### Example 4

##### [(S)-HOCH<sub>2</sub>CH(OH)C(O)]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>

By following the procedure of section c) of example 1, Boc-Val-ACHPA-Leu-Phe-NH<sub>2</sub> (81 mg, 0.12 mmol) was deprotected in 6N HCl/dioxane to give H-Val-ACHPA-Leu-Phe-NH<sub>2</sub> hydrochloride which was then coupled to L-glyceric acid hemicalcium salt dihydrate (Sigma Chemical Co., St. Louis, MO, USA, 21 mg, 0.14 mmol) with BOP (64 mg, 0.14 mmol) to give a crude product. Chromatography of crude product on silica gel (20 mL), using 1 to 15% gradients of MeOH in chloroform, followed by trituration of the resulting solid with Et<sub>2</sub>O afforded the title compound (33 mg, 42%). Mass spectrum: 662 (M+H)<sup>+</sup>. Amino acid analysis: ACHPA, 0.83; Leu, 1.02; Phe, 1.01; Val, 0.97.

The following compounds of formula 1 were prepared by following the procedure of example 1 and substituting the appropriate acid of formula X-OH and/or the appropriate amino acid derivatives representing the units A, B, C', D and Y.

#### Example 5:

Ac-Val-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 616 (M+H)<sup>+</sup>, amino acid analysis: ACHPA, 0.93; Leu, 1.25; Phe, 1.00; Val, 0.99.

#### Example 6:

PhCH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 706 (M+H)<sup>+</sup>, amino acid analysis: Leu,

0.98; Phe, 1.01; Val, 1.01.

Example 7:

- 5       $\text{PhCH}_2\text{CH}_2\text{CH}_2\text{C(O)-Val-ACHPA-Leu-Phe-NH}_2$ , mass spectrum: 720 (M+H)<sup>+</sup>, amino acid analysis: Leu, 0.95; Phe, 1.03; Val, 1.01.

Example 8:

- 10       $\text{PhCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{C(O)-Val-ACHPA-Leu-Phe-NH}_2$ , mass spectrum: 734 (M+H)<sup>+</sup>, amino acid analysis: Leu, 0.95; Phe, 1.04; Val, 1.01.

Example 9:

- 15      [(S)-CH<sub>3</sub>CH(OH)C(O)]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 646 (M+H)<sup>+</sup>, amino acid analysis: Leu 0.96; Phe, 1.05; Val, 0.98.

Example 10:

- 20      (4-MeOPh)CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 736 (M+H)<sup>+</sup>, amino acid analysis: Leu, 0.98; Phe, 1.06; Val, 0.96.

Example 11:

- 25      Ac-Tbg-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 630 (M+H)<sup>+</sup>, amino acid analysis: ACHPA, 0.7; Leu, 0.95; Phe, 1.05; Tbg, 1.0.

Example 12

- 30      Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu-Ile-NH<sub>2</sub>

a) Boc-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester

- Sodium cyanoborohydride (2.83 g, 45.0 mmol) was added portionwise over 15 min to a stirred solution of H-Leu-OH p-nitrobenzyl ester hydrochloride (10.36 g, 36.3 mmol) and Boc-phenylalaninal (15.06 g, 70.0 mmol), described by D.H. Rich and E.T.O. Sun, *supra*, in MeOH (250 ml). The resulting mixture was stirred for 18 h. The reaction mixture was added dropwise to a stirred, ice-cold saturated aqueous solution of NaHCO<sub>3</sub> (2.5 L). The aqueous solution was extracted twice with EtOAc. The combined organic extracts were washed three times with a saturated aqueous solution of NaCl, dried (MgSO<sub>4</sub>) and concentrated. The residue was triturated with hexane to give a solid. The solid was purified by chromatography on silica gel (eluent: EtOAc-hexane, 1:4) to give the desired product as a white solid (12.33 g, 76%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) 0.85-0.95 (6H, m), 1.3-1.85 (12H, m), 2.3-2.75 (2H, m), 2.75-2.9 (2H, m), 3.3 (2H, t), 3.7-3.9 (1H, m), 4.5-4.7 (1H, m), 5.2 (2H, s), 7.1-7.4 (5H, m), 7.45-8.25 (4H, 2d).

- 45      b) H-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester

- A solution of Boc-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester (11.6 g, 26 mmol) in 8N HCl/dioxane (90 mL) was stirred at room temperature under a nitrogen atmosphere for 30 min. The solvent was evaporated and the residue dried in high vacuum to afford H-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester hydrochloride (10.9 g, 100%).

c) Boc-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester

- The preceding product (5 g, 12 mmol), Boc-Val-OH (2.86 g, 13.2 mmol) and N-methylmorpholine (3.95 ml, 36 mmol) were dissolved in dimethylformamide (50 mL). This solution was adjusted to pH 8 (wet pH paper) by the addition of N-methylmorpholine. BOP (6.36 g, 14.4 mmol) was added to the solution. After 15 min, the pH of the reaction mixture was adjusted again to pH 8. The mixture was stirred for 18 h. Thereafter, the mixture was diluted with Et<sub>2</sub>O and washed successively with water, a saturated aqueous

solution of NaHCO<sub>3</sub> (three times) and a saturated aqueous solution of NaCl (two times). The organic phase was concentrated under reduced pressure. Chromatography of the residue on silica gel, using EtOAc-hexane (1:3) as the eluent, gave Boc-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester (4.8 g, 69 %) as a white solid.

5

d) Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH nitrobenzyl ester

By repeating the deprotection and coupling procedures of the preceding sections b) and c), Boc-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester (1.0 g, 1.7 mmol) was deprotected and coupled with acetic acid (114 mg, 1.9 mmol) to afford Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH p-nitrobenzyl ester (692 mg, 73%) as a white solid.

10

e) Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH

The preceding product (392 mg, 0.704 mmol) was dissolved in MeOH (5 mL). Oxygen was removed from the solution with a stream of dry nitrogen. Under an atmosphere of nitrogen, 10% by weight of palladium on charcoal (5 mg) was added to the solution. The mixture was stirred under an atmosphere of hydrogen for 4 h. The mixture was filtered through diatomaceous earth and the filtrate was concentrated to dryness. Trituration of the residue with Et<sub>2</sub>O gave Ac-Pheψ[CH<sub>2</sub>NH]Leu-OH (241 mg, 81%) as a white solid.

20

f) Boc-Glu(OBzl)-Ile-NH<sub>2</sub>

The preceding deprotection and coupling procedures of sections b) and c) of this example were used to assemble Boc-Glu(OBzl)-Ile-NH<sub>2</sub> in a quantitative yield by deprotecting Boc-Ile-NH<sub>2</sub> and coupling the resulting H-Ile-NH<sub>2</sub> hydrochloride with Boc-Glu(OBzl)-OH.

25

g) Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu(OBzl)-Ile-NH<sub>2</sub>

Again by using the deprotection coupling procedures of this example, deprotection of Boc-Glu(OBzl)-Ile-NH<sub>2</sub> and coupling of the resulting H-Glu(OBzl)-Ile-NH<sub>2</sub> hydrochloride (24.6 mg, 0.066 mmol) with Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-OH (28 mg, 0.066 mmol) afforded a crude product. The crude product was purified by reverse phase HPLC on a octadecasilyl-silica column (H<sub>2</sub>O [0.06% TFA]-acetonitrile [0.06% TFA] gradient). In this manner, Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu(OBzl)-Ile-NH<sub>2</sub> (25.3 mg, 52%) was obtained as a white solid.

30

h) Title compound of this example

35

The preceding product (25.3 mg, 0.034 mmol) was subjected to hydrogenolysis according to the procedure of section e) of this example to afford Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu-Ile-NH<sub>2</sub> (21.2 mg, 96%). Mass spectrum: 647 (M + H)<sup>+</sup>. Amino acid analysis: Glu, 1.01; Val, 0.59; Phe, 0.99 (Pheψ[CH<sub>2</sub>NH]Leu does not hydrolyze and Val-Pheψ[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

40

The following compounds of formula 1 were prepared by following the procedure of example 12 and using the appropriate amino acid derivatives.

Example 13:

45

Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 646 (M + H)<sup>+</sup>, amino acid analysis: Glx, 1.02; Ile, 0.98; Val, 0.55 (Val-Phe[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

Example 14:

50

Ac-Tbg-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 660 (M + H)<sup>+</sup>, amino acid analysis: Glx, 0.96; Ile, 1.04.

Example 15:

55

Ac-Ala-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 618 (M + H)<sup>+</sup>, amino acid analysis: Glx, 1.05; Ala, 0.99; Ile, 0.96.

Example 16:

Ac-Ile-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 660 (M+H)<sup>+</sup>, amino acid analysis: Glx, 1.00; Ile, 1.28.

5

Example 17:

Ac-Cpg-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 672 (M+H)<sup>+</sup>.

10 Example 18:

(CH<sub>3</sub>)<sub>2</sub>CHC(O)-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 674 (M+H)<sup>+</sup>, amino acid analysis: Glx, 1.02; Val, 0.98; Ile, 1.00.

15 Example 19:

[(S)-CH<sub>3</sub>CH(OH)C(O)]-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 676 (M+H)<sup>+</sup>, amino acid analysis: Glx, 1.01; Val, 0.30; Ile, 0.99. (Val-Pheψ[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

20 Example 20:

MeOC(O)-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>, mass spectrum: 662 (M+H)<sup>+</sup>, amino acid analysis: Glx, 0.98; Val, 0.25; Ile, 1.02. (Val-Phe[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

25 Example 21:

Ac-Val-Pheψ[CH<sub>2</sub>NH]Tba-Gln-Ile-NH<sub>2</sub>, mass spectrum: 660 (M+H)<sup>+</sup>.

Example 22:

30

Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Val-Ser(OBzl)-NH<sub>2</sub>, mass spectrum: 681 (M+H)<sup>+</sup>, amino acid analysis: Ser, 1.00; Val, 1.23 (Val-Pheψ[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

Example 23:

35

Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Val-Ile-NH<sub>2</sub>, amino acid analysis: Val, 1.45; Ile, 1.00 (Val-Pheψ[CH<sub>2</sub>NH]Leu only partially hydrolyzes).

Example 24:

40

[(S)-PhCH<sub>2</sub>CH(OH)CO]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>, mass spectrum: 722 (M+H)<sup>+</sup>; amino acid analysis: Leu, 1.14; Phe, 1.02; Val, 0.98.

Other examples of compounds of formula 1 that can be made by the preceding procedures are:

CH<sub>3</sub>CH<sub>2</sub>CO-Ala-Tyrψ[CH<sub>2</sub>NH]Ile-Glu-Ser(OBzl)-OMe

45 (4-CH<sub>3</sub>Ph)CH<sub>2</sub>CH<sub>2</sub>CO-Ile-Pheψ[CH<sub>2</sub>NH]Cpa-Glu-Phe-O(CH<sub>2</sub>)<sub>4</sub>CH<sub>3</sub>

CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CO-Ile-Sta-Glu-Ser(OBzl)-NHCH<sub>3</sub>

PhCH<sub>2</sub>CH<sub>2</sub>CO-Ile-ACCPA-Glu-Phe-OCH<sub>2</sub>CH<sub>3</sub>

Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu(OMe)-Ile-NH<sub>2</sub>

CH<sub>3</sub>CH<sub>2</sub>CO-Val-ACHPA-Cha-Phe-NH<sub>2</sub>

50 PhCH<sub>2</sub>CH<sub>2</sub>CO-Val-AHPPA-Cpa-Phe-NH<sub>2</sub>Example 25

Recombinant HIV Protease HPLC Assay:

55 Enzyme: HIV protease was expressed in E. coli [construct pBRT1 prt<sup>+</sup>, see W.G. Farmerie et al., Science, 236, 305 (1987)], according to the following protocol:

Unless stated otherwise, all solutions are aqueous solutions.

(i) Fermentation

E. coli cells containing the pBRT1 prt<sup>+</sup> plasmid were used to inoculate a seed culture media composed of Luria-Bertani Broth containing 100 µg/mL of ampicillin. Flasks were incubated at 37° under agitation for 17 h. Production flasks containing sterile M9 broth, supplemented with 100 µg/ml of ampicillin, were inoculated at a level of 1% (v/v) using the seed culture described above. Total volume in each production flask was 500 mL in a 2 L Erlenmeyer flask. Flasks were incubated at 37° under agitation until a cell concentration corresponding to an optical density ( $\lambda = 540$  m) of 0.6 was reached (no dilution). This time span was routinely 3-4 h. Flasks were then supplemented with 5mM isopropylthiogalactoside (IPTG, Research Organics, Cleveland, Ohio, USA) and incubation was continued until the cell concentration reached an optical density of 0.2 at 16-fold dilution. Flasks were then supplemented with 1mM phenylmethylsulfonyl fluoride (PMSF) and rapidly chilled to 4°. The bacterial cells were recovered by centrifugation at 4°. The wet pellet was stored at -70°.

(ii) Extraction and Preparation of Assay Grade Enzyme:

All steps below were performed at 4° unless otherwise indicated. Thawed cells were mixed with buffer A [50 mM tris(hydroxymethyl)aminoethane HCl (Tris-HCl, pH 7.4); 0.6 mM ethylenediaminetetraacetic acid (EDTA); 0.375 M NaCl; 0.2% Nonidet P-40<sup>R</sup> (BDH Chemicals Ltd., Poole, UK); 1mM PMSF], at a ratio of one part cells to nine parts buffer A. Diatomaceous earth (Celite 545<sup>R</sup>, John Manville, Lompoc, CA, USA) was added at a ratio of two parts to one part of wet cell weight. The resulting slurry was homogenized at high speed (ca. 20,000 rpm) on a Waring<sup>R</sup> Industrial blender for 8 x 15 sec. pulses. Cell debris/Celite<sup>R</sup> was collected by centrifugation and the resulting pellet was extracted with 4.5 parts of buffer A to one part wet solids using the homogenization procedure described above. Supernatants from both homogenization steps were combined and soluble protein was precipitated by the addition of solid (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to yield a final concentration of 75% saturation. This mixture was agitated for 60 min and the precipitate was recovered by centrifugation. The resulting pellet was suspended in buffer B [50mM Tris-HCl, pH 8; 30 mM NaCl; 1 mM DL-dithio-threitol (DTT); 1 mM EDTA; 1 mM PMSF; 10% glycerol], and dialyzed for 18h against the same buffer.

An aliquot of the dialyzed extract containing 150 mg of the protein was loaded onto a Sephadex A25<sup>R</sup> anion exchange column. (Pharmacia, Uppsala, Sweden) with bed dimensions of 70 cm length and 2.5 cm diameter. The sample was eluted isocratically with buffer B at a linear flow rate of 10 cm/h. Fractions containing HIV protease activity (see below for assay description) were combined, and soluble protein was precipitated by the addition of saturated aqueous (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> to yield a total (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> concentration of 85% saturation. Precipitated protein was removed by centrifugation and the resulting pellet was dissolved in buffer C [50 mM 2-(4-morpholino)ethanesulfonic acid (MES), pH 5.5; 150 mM NaCl; 1 mM DTT; 1 mM EDTA; 10% glycerol]. This preparation was dialyzed for 18h against buffer C, and then frozen at -70°. All crude extracts were purified by chromatography in aliquots containing 150 mg of protein in the same manner as described above. The final preparations from each batch were pooled, divided into 34 µL aliquots and stored at -70°. The final protein recovered from a 20 L fermentation was typically 300 mg with a specific activity for HIV protease of 18.2 mmoles of substrate cleaved/min/mg.

The aliquots were diluted to 1/38 of the original concentration with buffer, see below, prior to use (i.e. the enzyme working solution).

Substrate:

VSFNFPQITL-NH<sub>2</sub> MW 1164, see Krausslich et al., Proc. Natl. Acad. Sci. USA, 86, 807(1989), was used as substrate. The substrate was made into 10 mM stock in DMSO and stored at 4°. Prior to use, the stock was diluted with buffer to give a 400 µM solution (i.e. the substrate working solution).

Buffer:

MES (100 mM), KCl (300 mM) and EDTA (5 mM) were dissolved in distilled H<sub>2</sub>O (90 mL) and the solution was adjusted to pH 5.5 with concentrated aqueous NaOH. The latter solution was diluted to 100 mL with H<sub>2</sub>O to give the buffer.

Procedure:

(1) The assay mixture was prepared by mixing 20 $\mu$ L of the substrate working solution, 10 $\mu$ L of the solution of the test compound in 10% DMSO and 10 $\mu$ L of the enzyme working solution. (2) The assay mixture was incubated at 37° for 30 min. (3) The reaction was quenched by adding 200 $\mu$ L of 2% aqueous TFA. (4) The substrate and products (i.e. VSFNF and PQITL-NH<sub>2</sub>) were separated by subjecting 100 $\mu$ L of the quenched assay mixture to HPLC using a Perkin-Elmer 3 x 3 CRC8 column (Perkin Elmer Inc., Norwalk, CT, USA) with a stepwise gradient at a flow rate of 4 mL/min. The gradient is as follows:

0 - 0.5 minutes, 70% A / 30% B;

0.5 - 3.0 minutes, 67% A / 33% B;

3.0 - 5.0 minutes, 20% A / 80% B;

5.0 - 6.5 minutes, 70% A / 30% B;

where A is 3 mM sodium dodecyl sulfate 0.05% H<sub>3</sub>PO<sub>4</sub> in H<sub>2</sub>O and B is 0.05% H<sub>3</sub>PO<sub>4</sub> in acetonitrile. Elution was monitored at 210 nm. (5) A control, which was the assay mixture without the test compound, was subjected simultaneously to steps 2 to 4.

#### 15 Inhibition Studies:

Cleavage products and remaining parent substrate were quantified by either peak height or by integration of the appropriate HPLC peaks. Substrate conversion was calculated using the following relationship:

$$\% \text{ Conversion} = \frac{\text{Sum of peak height or peak area of products} \times 100}{\text{Sum of peak height or peak area of substrate and products}}$$

Enzyme inhibition of the test compound was calculated as follows:

$$\% \text{ Inhibition} = 100 - \frac{\% \text{ Conversion for assay mixture} \times 100}{\% \text{ Conversion of control}}$$

The concentration of the test compound which causes a 50% inhibition of the HIV-protease, i.e. the IC<sub>50</sub>, was determined as follows: The percent inhibition of the enzyme was determined for a minimum of three different concentrations of the test compound. Thereafter, the IC<sub>50</sub> was determined graphically by plotting the percent inhibition of the enzyme against the concentration of the test compound.

The following table of exemplified compounds of formula 1 lists their IC<sub>50</sub> as determined in the recombinant HIV protease HPLC assay.

5	Compound	Example in which compound is prepared	IC <sub>50</sub> (nM)
10	[4-(BzlOC(O)CH <sub>2</sub> O)Ph]CH <sub>2</sub> CH <sub>2</sub> C(O)-Val-ACHPA-Leu-Phe-NH <sub>2</sub>	2	170
15	[(S)-HOCH <sub>2</sub> CH(OH)C(O)]-Val-ACHPA-Leu-Phe-NH <sub>2</sub>	4	180
20	Ac-Val-ACHPA-Leu-Phe-NH <sub>2</sub>	5	280
25	Ac-Tbg-ACHPA-Leu-Phe-NH <sub>2</sub>	11	200
30	Ac-Val-Pheψ[CH <sub>2</sub> NH]Leu-Glu-Ile-NH <sub>2</sub>	12	6.7
35	Ac-Ile-Pheψ[CH <sub>2</sub> NH]Leu-Gln-Ile-NH <sub>2</sub> .TFA	16	180
40	(S)-CH <sub>3</sub> CH(OH)C(O)-Val-Pheψ[CH <sub>2</sub> NH]Leu-Gln-Ile-NH <sub>2</sub> .TFA	19	660
45	Ac-Val-Pheψ[CH <sub>2</sub> NH]Tba-Gln-Ile-NH <sub>2</sub> .TFA	21	720
50	Ac-Val-Pheψ[CH <sub>2</sub> NH]Leu-Val-Ser(OBzl)-NH <sub>2</sub>	22	29

Example 26

55 The following protocol, used for screening antiviral effects of the compounds of formula 1, is adapted from a plaque assay utilizing HTLV-I transformed cells which was developed by Harada et al., supra. HTLV-I transformed cells are used in these assays because of the rapidity with which HIV will replicate in these cells.

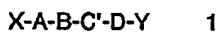
1. The test compound is dissolved in dimethylsulfoxide to a concentration of 5 mg/mL. The resultant solution can be stored at 4° until used.
2. The resultant solution is diluted in RPMI 1640 (Gibco Laboratories, St. Lawrence, MA, USA) to four times (4x) the final concentration which is to be tested. Once diluted in RPMI 1640, the solution is used in the cell culture assay within 4 h.
3. The 4x solution (50 $\mu$ L) is added to triplicate wells of a 96 well flat bottomed microtiter plate. RPMI 50 $\mu$ L also is added to control wells.
4. C8166 cells (5 x 10<sup>-4</sup>) in 50 L of HEPES-buffered RPMI 1640, 10% heat inactivated fetal calf serum (FCS), 12.5 $\mu$ L/ml gentamicin (complete media) are added to all wells.
5. Fifty times TCID<sub>50</sub> of H9/HTLV-III<sub>B</sub> stock (stored in liquid nitrogen as cell culture supernatant in 50% FCS) in 100 $\mu$ L of complete media is added to all wells. Infectious titer of virus stocks are as previously determined by end point dilution on C8166 cells. Titer of stocks are stable for 6-12 months when stored at -193°.
6. Microtiter plates are then placed on level shelves of a 37°, 5% CO<sub>2</sub> humidified incubator for 72 h.
7. Plates are then removed and centers of syncytia are counted in each well by low power phase contrast light microscopy. Each cluster of cells which shows evidence of any syncytia formation is counted as one center of syncytia. Control wells should have between 25 and 75 centers of syncytia per well.
8. Percent inhibition of syncytia formation is calculated by the formula:

$$\% \text{ inhibition} = 100 \times \frac{\left( \frac{\# \text{ syncytial centers}}{\text{in control wells}} \right) - \left( \frac{\# \text{ syncytial centers in}}{\text{test wells}} \right)}{\frac{\# \text{ syncytial center in}}{\text{control wells}}}$$

When tested according to this procedure, Ac-Val-ACHPA-Leu-Phe-NH<sub>2</sub> of example 5 and Ac-Tbg-ACHPA-Leu-Phe-NH<sub>2</sub> of example 11 exhibited 97% and 72% Inhibition at 20 $\mu$ g/mL.

#### Claims

1. A compound of formula 1



wherein X is

- (i) lower alkanoyl,
  - (ii) lower alkanoyl monosubstituted with a hydroxy,
  - (iii) alkanoyl, containing three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on different carbon atoms thereof,
  - (iv) (lower alkoxy)carbonyl,
  - (v) phenyl(lower alkanoyl),
  - (vi) phenyl(lower alkanoyl) monosubstituted on the alkanoyl portion thereof with hydroxy, or
  - (vii) phenyl(lower alkanoyl) monosubstituted at position 4 on the aromatic portion thereof with hydroxy, halo, lower alkyl, lower alkoxy, or OCH<sub>2</sub>C(O)OL wherein L is hydrogen, lower alkyl or benzyl;
- A is NHCH(R<sup>1</sup>)CO wherein R<sup>1</sup> is lower alkyl or lower cycloalkyl;
- B is NHCH(R<sup>2</sup>)-Q wherein R<sup>2</sup> is
- (i) lower alkyl,
  - (ii) lower cycloalkyl,
  - (iii) (lower cycloalkyl)methyl,
  - (iv) phenyl or phenyl monosubstituted with hydroxy, lower alkyl or lower alkoxy, or
  - (v) benzyl or benzyl monosubstituted on the aromatic portion thereof with hydroxy, lower alkyl or lower alkoxy, and
- Q is CH(OH)CH<sub>2</sub>C(O) or CH<sub>2</sub>NHCH(R<sup>3</sup>)C(O) wherein R<sup>3</sup> has the same meaning as defined for R<sup>2</sup> in this claim;



C' is  $\text{NHCH(R}^4\text{)C(O)}$  wherein  $\text{R}^4$  is lower alkyl, (lower cycloalkyl)methyl,  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{C(O)-T}$  or  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{CR}^9\text{R}^{10}\text{C(O)-T}$  wherein  $\text{R}^5$  to  $\text{R}^{10}$ , inclusive, are each independently a hydrogen or lower alkyl and T is hydroxy, lower alkoxy or amino;

D is  $\text{NHCH(R}^{11}\text{)C(O)}$  wherein  $\text{R}^{11}$  is phenylmethyl, lower alkyl, or lower alkyl monosubstituted with hydroxy or benzyloxy; and

Y is hydroxy, lower alkoxy, amino, lower alkylamino or di(lower alkyl)amino; or a therapeutically acceptable salt thereof.

2. A compound as recited in claim 1 wherein X is as defined in claim 1, A is Ala, Abu, Val, Nva, Leu, Ile, Tbg or Cpg; B is  $\text{NHCH(R}^2\text{)-Q}$  wherein  $\text{R}^2$  is lower alkyl, (lower cycloalkyl)methyl, benzyl, (4-hydroxyphenyl)methyl or (4-methoxyphenyl)methyl and Q is  $\text{CH(OH)CH}_2\text{C(O)}$  or  $\text{CH}_2\text{NHCH(R}^3\text{)C(O)}$  wherein  $\text{R}^3$  has the same meaning as defined for  $\text{R}^2$  in this claim; C' is  $\text{NHCH(R}^4\text{)C(O)}$  wherein  $\text{R}^4$  is lower alkyl, (lower cycloalkyl)methyl,  $\text{CR}^5\text{R}^6\text{CR}^7\text{R}^8\text{C(O)-T}$  wherein  $\text{R}^5$  to  $\text{R}^8$ , inclusive, are each hydrogen and T is hydroxy, methoxy or amino; D is Phe, Ile or Ser(OBzl); and Y is lower alkoxy or amino; or a therapeutically acceptable salt thereof.
3. A compound as recited in claim 2 wherein X is as defined in claim 2; A is Ala, Val, Leu, Ile or Tbg, B is  $\text{NHCH(R}^2\text{)CH(OH)CH}_2\text{C(O)}$  wherein  $\text{R}^2$  is 2-methylpropyl, cyclopropylmethyl, cyclohexylmethyl or benzyl, C' is Val or Leu, D is Phe and Y is amino; or a therapeutically acceptable salt thereof.
4. A compound as recited in claim 2 wherein X is lower alkanoyl; lower alkanoyl monosubstituted with hydroxy; alkanoyl, containing three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on a different carbon atom thereof; or (lower alkoxy)carbonyl; A is Ala, Val, Leu, Ile, Tbg or Cpg; B is  $\text{NHCH(R}^2\text{)CH}_2\text{NHCH(R}^3\text{)C(O)}$  wherein  $\text{R}^2$  is benzyl and  $\text{R}^3$  is lower alkyl; C' is Val, Glu or Gln; D is Ile or Ser(OBzl) and Y is amino; or a therapeutically acceptable salt thereof.
5. A compound as recited in claim 3 wherein X is acetyl, (S)-2-hydroxy-1-oxopropyl, (S)-2,3-dihydroxy-1-oxopropyl, (S)-2-hydroxy-1-oxo-3-phenylpropyl, 1-oxo-3-phenylpropyl, 1-oxo-4-phenylbutyl, 1-oxo-5-phenylpentyl, 3-(4-hydroxyphenyl)-1-oxopropyl, 3-(4-methoxyphenyl)-1-oxopropyl, 3-[4-(carboxymethoxy)phenyl]-1-oxopropyl or 3-[4-(benzyloxycarbonylmethoxy)phenyl]-1-oxopropyl, A is Val, Leu, Ile or Tbg, B is  $\text{NHCH(R}^2\text{)CH(OH)CH}_2\text{CO}$  wherein  $\text{R}^2$  is cyclohexylmethyl, C' is Leu, D is Phe and Y is amino; or a therapeutically acceptable salt thereof.
6. A compound as recited in claim 4 wherein X is acetyl, 2-methyl-1-oxopropyl, (S)-2-hydroxy-1-oxopropyl or methoxycarbonyl, A is Ala, Val, Ile, Tbg or Cpg, B is  $\text{NHCH(R}^2\text{)CH}_2\text{NHCH(R}^3\text{)C(O)}$  wherein  $\text{R}^2$  is benzyl and  $\text{R}^3$  is 2-methylpropyl or 2,2-dimethylpropyl, C' is Val, Glu or Gln, D is Ile or Ser(OBzl) and Y is amino; or a therapeutically acceptable salt thereof.
7. A compound as recited in claim 1 selected from the group consisting of:
  - (4-HOPh) $\text{CH}_2\text{CH}_2\text{CO-Val-ACHPA-Leu-Phe-NH}_2$ ,
  - [4-(BzlOC(O)CH<sub>2</sub>O)Ph] $\text{CH}_2\text{CH}_2\text{C(O)-Val-ACHPA-Leu-Phe-NH}_2$ ,
  - [4-(HOC(O)CH<sub>2</sub>O)Ph] $\text{CH}_2\text{CH}_2\text{C(O)-Val-ACHPA-Leu-Phe-NH}_2$ ,
  - [(S)-HOCH<sub>2</sub>CH(OH)CO]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>,
  - Ac-Val-ACHPA-Leu-Phe-NH<sub>2</sub>,
  - PhCH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>
  - PhCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>
  - PhCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>C(O)-Val-ACHPA-Leu-Phe-NH<sub>2</sub>,
  - [(S)-CH<sub>3</sub>CH(OH)C(O)]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>,
  - (4-MeOPh) $\text{CH}_2\text{CH}_2\text{C(O)-Val-ACHPA-Leu-Phe-NH}_2$ ,
  - Ac-Tbg-ACHPA-Leu-Phe-NH<sub>2</sub>,
  - Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Glu-Ile-NH<sub>2</sub>,
  - Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - Ac-Tbg-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - Ac-Ala-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - Ac-Ile-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - Ac-Cpg-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - (CH<sub>3</sub>)<sub>2</sub>CHC(O)-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,
  - [(S)-CH<sub>3</sub>CH(OH)C(O)]-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,

MeOC(O)-Val-Pheψ[CH<sub>2</sub>NH]Leu-Gln-Ile-NH<sub>2</sub>,  
 Ac-Val-Pheψ[CH<sub>2</sub>NH]Tba-Gln-Ile-NH<sub>2</sub>,  
 Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Val-Ser(OBzl)-NH<sub>2</sub>,  
 Ac-Val-Pheψ[CH<sub>2</sub>NH]Leu-Val-Ile-NH<sub>2</sub>, and  
 [(S)-PhCH<sub>2</sub>CH(OH)CO]-Val-ACHPA-Leu-Phe-NH<sub>2</sub>.

8. A pharmaceutical composition comprising a compound as recited in claim 1, or a therapeutically acceptable salt thereof, and a pharmaceutically acceptable carrier.
9. The use of a compound as recited in anyone of claims 1 to 7, or a therapeutically acceptable salt thereof, for the manufacture of a medicament for treating HIV infections in a human.
10. The use of a compound as recited in anyone of claims 1 to 7, or a therapeutically acceptable salt thereof, for the manufacture of a medicament for protecting human cells against HIV pathogenesis.
11. A process for preparing a compound of formula 1 as recited in claim 1 comprising:
  - (i) coupling an acid of the formula X<sup>1</sup>-OH wherein X<sup>1</sup> is lower alkanoyl; a lower alkanoyl monosubstituted with a hydroxy; an alkanoyl of three to six carbon atoms, disubstituted with hydroxy, each hydroxy being on different carbon atoms thereof; a phenyl(lower alkanoyl); a phenyl(lower alkanoyl) monosubstituted on the alkanoyl portion thereof with a hydroxy; or a phenyl(lower alkanoyl) monosubstituted at position 4 on the aromatic portion thereof with hydroxy, lower alkyl, lower alkoxy or OCH<sub>2</sub>OC(O)-OL<sup>1</sup> wherein L<sup>1</sup> is lower alkyl or benzyl; with an intermediate of formula H-A-B-C''-D-Y wherein A, B, D and Y are as defined in claim 1 and C'' is the radical NHCH(R<sup>4A</sup>)CO wherein R<sup>4A</sup> is lower alkyl, (lower cycloalkyl)methyl, CR<sup>5</sup>R<sup>6</sup>CR<sup>7</sup>R<sup>8</sup>C(O)-T<sup>1</sup> or CR<sup>5</sup>R<sup>6</sup>CR<sup>7</sup>R<sup>8</sup>CR<sup>9</sup>R<sup>10</sup>C(O)-T<sup>1</sup> wherein R<sup>5</sup> to R<sup>10</sup>, inclusive, are as defined in claim 1 and T<sup>1</sup> is an O-(carboxy protective group) or amino; or
  - ii) subjecting the intermediate of formula X<sup>1</sup>-A-B-C''-D-Y wherein X<sup>1</sup> is a phenyl(lower alkanoyl) monosubstituted at position 4 on the aromatic portion thereof with hydroxy to O-alkylation with an alkylating agent of formula K-CH<sub>2</sub>C(O)-OL<sup>1</sup> wherein K is bromo, chloro or iodo and L<sup>1</sup> is as defined in this claim, in the presence of a suitable strong base;
 followed, if required, by carboxy deprotection or by carboxy deprotection and esterification to obtain the corresponding compound of formula 1; and, if desired, transforming the peptide into a therapeutically acceptable salt.



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⑤④ HIV protease inhibiting agents.

57 Disclosed herein are compounds of the general formula X-A-B-C'-D-Y wherein X is a terminal group, for example, alkanoyl or phenylalkanoyl radicals, A is an amino acid residue having a lower alkyl or lower cycloalkyl side chain, e.g. Ala or val, B is a non-peptide linking unit, e.g. statyl or Pheψ[CH<sub>2</sub>NH]Leu, C' is an amino acid residue of α-amino-β-cycloalkyl-propionic acid, glutamic acid or α-aminoadipic acid, or a related derivative thereof, D is an amino-acid residue, for example, Phe or Val, and Y is hydroxy, alkoxy, amino, alkylamino or dialkylamino. The compounds inhibit the activity of human immunodeficiency virus (HIV) protease and interfere with HIV induced cytopathogenic effects in human cells. These properties render the compounds used for combating HIV infections.

**EP 0 443 559 A3**



European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

DOCUMENTS CONSIDERED TO BE RELEVANT			EP 91102496.6
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P, X	<u>EP - A - 0 374 097</u> (CIBA-GEIGY AG) * Claims 1-5 * --	1-11	C 07 K 5/02 C 07 K 7/02 A 61 K 37/64 A 61 K 37/02
P, X	<u>EP - A - 0 357 332</u> (MERCK & CO INC.) * Claims 1-3 * --	1-11	C 07 K 1/00
X	<u>EP - A - 0 352 000</u> (SMITHKLINE BECKMAN CORP.) * Claim 1 * ----	1-11	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C 07 K 5/00
The present search report has been drawn up for all claims			
Place of search VIENNA		Date of completion of the search 13-04-1992	Examiner SCHARF
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			